Use of Human Monoclonal Antibodies to Treat Chikungunya Virus Infection

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Chikungunya virus (CHIKV) is an alphavirus prevalent in tropical regions. It causes an acute febrile disease that, in elderly individuals and newborns, is often associated with severe complications. We previously reported the isolation and characterization of 2 human monoclonal antibodies neutralizing CHIKV in vitro: 5F10 and 8B10. Here, we tested their efficacy in vivo as prophylactic and therapeutic treatments of CHIKV infection in AGR129 mice. In both settings, 5F10 and 8B10 were able to significantly delay CHIKV-driven lethality. Our results support the development of prophylactic and therapeutic treatments for CHIKV infection, using a combination of 5F10 and 8B10.

**Keywords.** monoclonal antibodies; Chikungunya virus; protection; in vivo; therapeutic; prophylactic.

Chikungunya virus (CHIKV) is an alphavirus of the Togaviridae family transmitted to humans by CHIKV-infected Aedes species mosquitoes [1, 2]. It is prevalent in tropical areas of Africa and Southeast Asia, but the recent spread of *Aedes albopictus* in temperate regions also puts Europe and Northern America at risk of disease outbreak [3, 4].

Acute human CHIKV infection is usually associated with dengue fever–like symptoms, (ie, fever, joint and muscle pain, and rash), which are often complicated by long-lasting and debilitating arthralgia [2, 5]. Newborns and elderly individuals are at high risk for severe complications [6].

Today, the treatment of the disease is purely symptomatic [7]. The use of anti-CHIKV polyclonal antibodies was shown to combat infection in mice [8]. We recently described 2 recombinant immunoglobulin G1 (IgG1) human monoclonal antibodies (mAbs), 5F10 and 8B10, that potently and specifically neutralize CHIKV in vitro by binding to CHIKV E1 and/or E2 envelope proteins [9, 10]. Here, we report the successful use of these 2 mAbs to protect against CHIKV infection in vivo. The present data pave the way to further clinical development of these mAbs as potential therapeutic tools for the prevention and treatment of human CHIKV infection.

**METHODS**

This study was performed in strict accordance with the guidelines of the Agri-Food and Veterinary Authority and the National Advisory Committee for Laboratory Animal Research of Singapore and was approved by the Institutional Animal Care and Use Committee of the Biological Research Center (Biomedical Sciences Institute, A*STAR).

AGR129 mice (interferon [-α/β/γR−/−/− and RAG-2−]) were described elsewhere [11] and further shown to be susceptible to CHIKV infection [10]. In the present study, mice aged 6–12 weeks were used.

CHIKV-specific IgG1 5F10 and 8B10 and the irrelevant human IgG1 HA4 (kindly provided by DSO National Laboratories, Singapore) have been described elsewhere [9].

Mice were infected with the CHIKV clinical isolate CHK/Singapore/07/2008 (also known as "CHIKV07"), kindly provided by Raymond T. P. Lin (National University Hospital, Singapore).

In vivo efficacy of 5F10 and 8B10 was assessed in both prophylactic and therapeutic settings.

For the prophylactic experiment, AGR129 mice were injected intraperitoneally with 250 µg (12.5 mg/kg) of 5F10 (n = 6), 8B10 (n = 6), 5F10 and 8B10 (125 µg of each; n = 5), or HA4 (n = 5), or with 25 µg (1.2 mg/kg) of 5F10 (n = 5), 8B10 (n = 4), or 5F10 and 8B10 (12.5 µg of each; n = 4). Six hours after mAb administration, mice were inoculated intravenously with 1000 CHIKV plaque-forming units (PFU).

For the therapeutic experiment, AGR129 mice were infected intravenously with 1000 CHIKV PFU 8 hours prior to intraperitoneal administration of 250 µg of 5F10 (n = 4), 8B10 (n = 5), 5F10 and 8B10 (12.5 µg of each; n = 4), or HA4 (n = 4).
Mice survival was assessed daily. Statistical analyses were performed with GraphPad Prism 5 using log-rank test (ie, Mantel-Cox).

To assess viral clearance, serum was collected from 10 surviving mice treated with the mAb combination at the 2 doses tested and in both prophylactic and treatment settings. Samples were taken near the end point, defined as 0–3 days before sacrifice (for the prophylactic setting) or as 5–11 days before death (for the treatment setting). Viral titers were measured by a plaque assay, using the parent CHIKV as a positive control and serum from 2 naïve AGR129 mice as a negative control.

RESULTS

We previously showed that 5F10 and 8B10 potently and specifically neutralize CHIKV in vitro [9]. Here, we investigated their capacity to protect and treat AGR129 mice against CHIKV infection.

The in vivo biological activity of mAbs 5F10 and 8B10 was initially evaluated in a prophylactic experiment. Two different mAbs doses, 25 µg or 250 µg/animal, were administrated to mice 6 hours prior to CHIKV infection. At a dose as low as 25 µg of mAb, prophylactic administration of 5F10 and/or 8B10 significantly delayed postinfection lethality (Figure 1). Indeed, the majority of 5F10- and/or 8B10-injected mice survived for at least 7 days after infection, when all irrelevant mAb-injected mice died within 3 days before infection (P = .0027 for 5F10 vs irrelevant; P = .0047 for 8B10 or 5F10 + 8B10 vs irrelevant; Figure 1). When administered at the higher dose of 250 µg, 5F10 and 8B10 induced a significant longer postinfection survival, compared with the lower dose, thus demonstrating a dose-dependent activity (P = .0011 for 8B10250 µg vs 8B1025 µg; P = .00705 for 5F10250 µg vs 5F1025 µg; Figure 1). Interestingly, although no statistical differences were observed between 5F10-, 8B10- or 5F10 + 8B10-treated mice at a dose of 25 µg/mouse, 8B10 conferred complete protection under the highest dose, with 100% of the mice surviving up to 20 days after infection (P = .0005 for 8B10250 µg vs 5F10250 µg; Figure 1). Altogether, a single injection of human anti-CHIKV IgG1 mAb at doses of 1.2 and 12 mg/kg conferred medium- to long-term protection against subsequent CHIKV infection. Moreover at high mAb dose, 8B10 was more protective than 5F10.

We next assessed the ability of 5F10 and/or 8B10 to treat a preexisting CHIKV infection. Mice were infected with CHIKV and 8 hours later received 250 µg of mAb. Mice injected with the irrelevant mAb died within 3–4 days after infection, while 5F10 or 8B10 treatment was associated with a significant 2–3-day delay in postinfection lethality (P = .0058 for 5F10 vs 8B10250 µg vs 5F10250 µg; Figure 1).

Figure 1. Survival of AGR129 mice after prophylactic administration of 5F10 and/or 8B10 followed by chikungunya virus (CHIKV) infection. AGR129 mice were injected with either 25 µg or 250 µg of 5F10 and/or 8B10, or with 250 µg of irrelevant monoclonal antibody as a negative control. Six hours later, mice were infected with CHIKV and observed daily. *P < .05, **P < .01, and ***P < .001, by the log-rank test (ie, Mantel-Cox).
irrelevant; \( P = .0082 \) for 8B10 vs irrelevant; \( P = .0058 \) for 5F10 + 8B10 vs irrelevant; Figure 2). Interestingly, unlike in the prophylactic experiment, the 5F10 and 8B10 combination conferred a significant 10-day longer protection than single mAbs (\( P = .0058 \) for 5F10 vs 5F10 + 8B10; \( P = .0046 \) for 8B10 vs 5F10 + 8B10; Figure 2), indicating a synergistic effect of the 2 mAbs under therapeutic conditions.

To confirm mAb efficacy with an independent readout, we measured viral titers in serum obtained near the end point from 10 surviving mice treated with the mAb combination, at both doses (25 and 250 \( \mu \)g) and in both prophylactic and treatment settings. Of the 10 mice tested, 9 were negative for CHIKV in serum, with only 1 showing detectable viremia (1.5 \( \times 10^5 \) PFU/mL; data not shown). Overall, these results suggest that the mAb combination has a long-term efficacy in controlling systemic viral dissemination.

**DISCUSSION**

This study describes the ability of 2 human mAbs, 5F10 and 8B10, to treat CHIKV infection in vivo both in prophylactic and therapeutic settings.

In the prophylactic experiment, we tested mAb doses in line with prescribed dosages for currently approved therapeutic human mAbs (ie, between approximately 1 and 15 mg/kg). The highest mAb dose conferred a long-lasting protection in up to 100% of the mice, while the low dose significantly delayed postinfection mortality. In the therapeutic settings, mAbs were also able to significantly delay lethality but did not fully protect from the outcome of infection. The observed delayed death of infected animals suggests a progressive loss of efficacy of the mAbs, possibly due to an unfavorable mAb/virus ratio. Two hypotheses could explain this unfavorable ratio: (1) a rapid clearance of the mAbs combined with the mode of dissemination of the virus and (2) the emergence of virus escaping the mAb-induced neutralization.

It was shown previously that human mAb injected in mice at doses comparable to those used in this study have a half-life of 6–10 days [12, 13]. Clearance of mAbs might result in a decrease of 5F10 and 8B10 titers below the protective threshold in a few days. The mAb clearance hypothesis would fit with the dose-dependent activity of the mAbs observed in the prophylactic experiment, as it would take longer for the highest dose of mAb to drop below a protective concentration. In addition to the clearance of mAbs, the persistence and replication of the virus in vivo in tissue niches may also explain the unfavorable mAb/virus ratio. We previously showed that, under extracellular neutralizing antibody pressure, CHIKV was able to disseminate by cell-to-cell transmission in vitro in human cells [10]. The strain we used in the present study is particularly prone to this mode of dissemination. Although cell-to-cell transmission has not been shown in vivo, should CHIKV be able to use this transmission mode in mice, it could escape neutralization by mAbs in the extracellular...
compartment. By use of cell-to-cell transmission, CHIKV could hide, disseminate to different niche organs, and constitute, there, reservoirs of viral particles that would be inaccessible to mAbs. Viral production could be reactivated within such niches once mAb titers drop to below protective levels.

However, the mAb clearance and CHIKV dissemination hypotheses cannot explain the longer protection conferred by the combination of 5F10 and 8B10 in the therapeutic settings. Indeed, the total amount of mAbs administered was the same as for single antibodies and one could therefore expect a similar clearer. RNA viruses, such as CHIKV, are prone to high nucleotide sequence modification [14]; it is possible that escape mutations that permitted evasion of mAb-dependent neutralization were selected during mAb treatment. Interestingly, we previously observed that even after several culture rounds under continuous mAb pressure, a CHIKV population fully escaping 8B10 and 5F10 + 8B10 was hardly selectable, whereas only a few selection rounds were sufficient to select a viral population that escaped 5F10 [10]. This suggested that 5F10 escape mutations were compatible with fit CHIKV replication, whereas those conferring resistance to 8B10 and 5F10 + 8B10 were not. In the present study settings, mAb concentrations were high, compared with those of our previous in vitro study. Consequently, CHIKV was under high selective pressure, and it is possible that resistant quasispecies were selected rapidly. In line with our previous data [10], we hypothesize that the 5F10 + 8B10 combination efficiently treats CHIKV infection because it does not lead to the emergence of 5F10 + 8B10-resistant CHIKV variants. The higher efficiency of 8B10 as compared to 5F10 (Figure 1) may also be due to the impaired fitness of viral variants escaping 8B10, when compared with those escaping 5F10 [10]. Additionally, it cannot be ruled out that, since 5F10 and 8B10 target different epitopes [10], they inhibit different stages of CHIKV cell infection (viral entry into host cell and viral-cell membranes fusion) and that concomitant disruption of various cell infection steps adds up to confer a higher protection. There is currently no available virus-specific treatment to fight human CHIKV infection. Although it may be costly and unpractical to protect significant portions of the population from acute CHIKV infection with an antibody-based drug, the mAbs might be useful in targeting restricted populations at risk of severe complications, such as pregnant women, newborns, elderly individuals, or individuals with preexisting severe arthritis. Together with our previously published in vitro data [9, 10], the in vivo results shown here encourage further development of the human mAbs 5F10 and 8B10 as candidate therapeutics against CHIKV infection. On the basis of current results, such a treatment should be based on a 5F10 + 8B10 combination, to counteract the selection of resistant variants, and possibly on a multiple-dose regimen, to maintain a mAb/virus ratio favorable to the mAbs.

Notes

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References